Evaluation of Prolonged Clotting Times

Lessons from the ARUP pathologist-directed panel

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Objectives

Describe appropriate assays to further evaluate prolonged PT and aPTT.

Evaluate benefits and challenges of pathologist contributions to test selection and interpretation in a reflexive prolonged clotting time panel.

Discuss patterns of results in prolonged clotting time panels performed and interpreted at ARUP Laboratories.

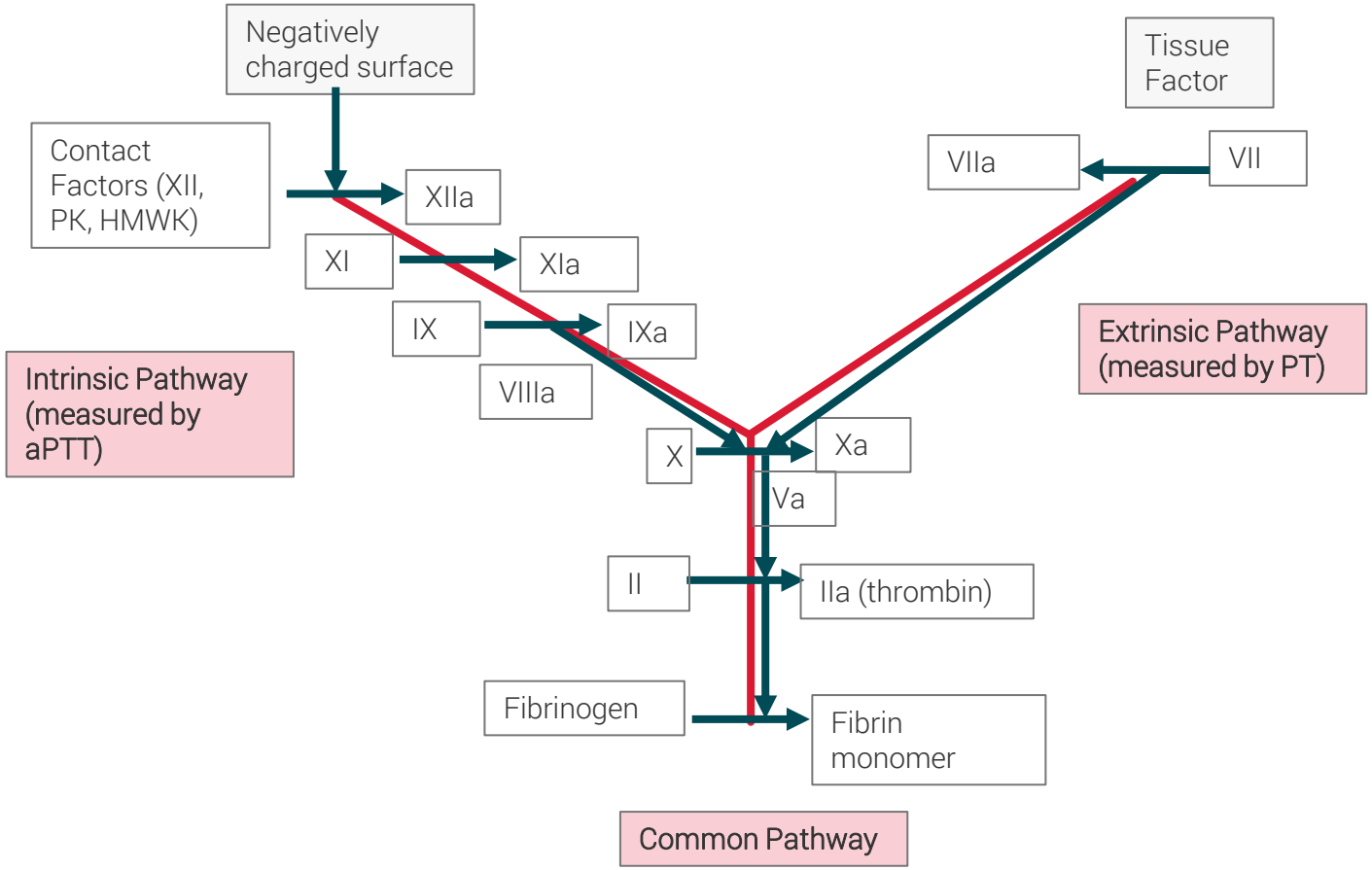
PT and aPTT prolongation

Basic strategies and clinical context

When are we asked to evaluate clotting time prolongations in the laboratory?

- Incidentally identified prolongations
- Bleeding patients with clotting time prolongations in the first round of diagnostic testing
- Evaluation for suspected acquired or inherited coagulation factor deficiency
- Anticoagulant therapy- monitoring and evaluation of unexpected excess prolongation for dose

Coagulation Cascade (simplified)



Algorithmic Approaches

- Increasingly discussed in literature in recent 10-15 years
- Focuses on value pathologist can add through consultation for test selection and interpretation
- Offer logical, reproducible patterns of test ordering based on understanding of coagulation cascade and clotting time assays
  - » Can be customized for each individual clinical context

Kottke-Marchant K. Semin Thromb Haemost. 2014; 40:195-204.
Rasmussen KL, Phillips M, Tripodi A, Goetze JP. Eur J Hematol. 2020; 104:519-525.
Hayward CPM. Int J Lab Hematol. 2018; 40 Suppl 1:6-14.

Differential Diagnosis of Prolonged PT

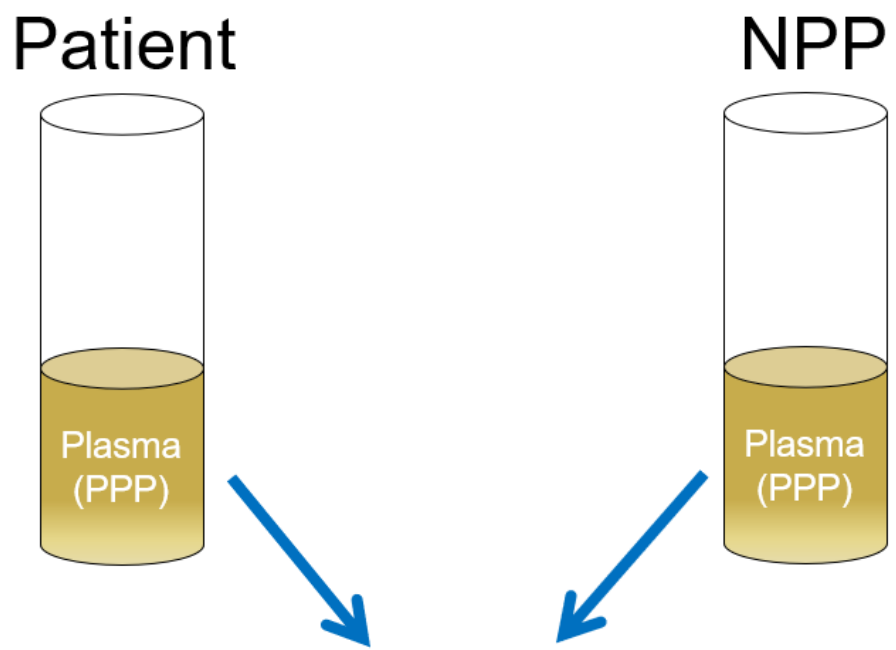
- Extrinsic factor deficiency or specific factor inhibitor (factor VII)
- Anticoagulant medications
  - Warfarin
  - Direct Xa inhibitors (degree of prolongation varies by reagent and drug)
- Liver disease
  - » Expect decreases in all factors except factor VIII
- Vitamin K deficiency
- Some cases of DIC
- Preanalytic variables

What about Lupus Anticoagulant?
Does not tend to prolong PT due to high amounts of phospholipid in PT reagent.*
(*except in cases of LA with associated hypoprothrombinemia)

Mixing Studies—Quick Review

1:1 mix = 1 part patient, 1 part NPP

4:1 mix = 4 parts patient, 1 part NPP



Interpretation

Corrects= Factor Deficiency Pattern
Does Not Correct= Inhibitor Pattern

Immediate Mix = measure PT or aPTT immediately

Incubated Mix* = incubate samples for 1-2 hours at 37°C, then measure aPTT

***Also includes incubation control (patient and NPP incubated separately and mixed at end of incubation, aPTT measured)**

Differential Diagnosis of Non-Corrected Mixing Study

PT	aPTT
Extrinsic factor inhibitor (VII)	Intrinsic factor inhibitor (VIII, IX, XI, contact factors)
Anticoagulants • Direct Xa inhibitors • Direct thrombin inhibitors (high levels) • Heparin (if it exceeds capacity of heparin neutralizer in PT reagent)	Anticoagulants • Heparin • Direct thrombin inhibitors • Direct Xa inhibitors (high levels)
	Lupus anticoagulant

Common pathway factor inhibitors (X, V, II) tend to prolong both PT and aPTT and show noncorrection in both PT and aPTT mixing studies.

Differential Diagnosis of prolonged aPTT

- Intrinsic factor deficiency or specific factor inhibitor
  - » Factors VIII, IX, XI; contact factors (XII, prekallikrein, high molecular weight kininogen)
- Lupus anticoagulant
- Anticoagulant medications, most notably
  - Heparin
  - Direct thrombin inhibitors
    - Argatroban
    - Dabigatran (variable prolongation with different reagents)
- Elevated CRP
- Some cases of von Willebrand disease (if factor VIII activity sufficiently low)
- Preanalytic variables

Differential Diagnosis of Prolonged PT and aPTT

- Common pathway factor deficiency or specific factor inhibitor
  - » Factors X, V, II, fibrinogen
- Severe liver disease
- DIC
- High levels of warfarin or superwarfarins / severe vitamin K deficiency
- High levels of heparin
- Lupus anticoagulant with hypoprothrombinemia (rare)
- Preanalytic variables
  - » Monoclonal proteins may sometimes cause unusual interference in clotting times

Strategies for Test Ordering

- Order everything up front
  - number of tests has greater risk of false positive results
- Order tests in step-wise fashion
  - additional phlebotomy episodes (and possibly exacerbated iatrogenic anemia), additional trips to lab for patient

What if you could combine these strategies to realize the benefits of both?

This is what the ARUP pathologist-directed prolonged clotting time panel aims to do.

How does ARUP's pathologist-directed prolonged clotting time panel work?

History and Structure of ARUP Panel

- Initially offered in 2017 in response to requests from clients
- Request form includes four simple history questions
  - » Describe the clinical presentation.
    - Bleeding / Thrombosis / Unexpected Clotting Time Prolongation
  - » Which clotting time is prolonged locally?
    - PT / aPTT / dRVVT / TT
  - » Anticoagulants and related drugs– date given if within past 7 days
    - Warfarin / UFH / Fondaparinux / Vitamin K / Thrombolytic / DTI / LMWH / DXa inh
  - » Any transfusions within past 72 hours?
    - DDAVP / Cryo / FFP / VWF, FVIII, or FIX concentrate / Other

ARUP Strategies to Detect Pre-Analytic Issues

- All specimens in our laboratory are evaluated to confirm correct specimen type (citrated plasma)
  - » Visual inspection for clots
  - » Screening clotting times (PT and/or aPTT)
  - » If clotting times are prolonged, additional studies may include
    - Fibrinogen activity to exclude serum
    - Sodium tetraphenylborate testing to exclude EDTA
      - › Forms a salt precipitate in specimens with high potassium that causes turbidity
      - › Alternate strategy– Ca^{2+} essentially undetectable, K^{+} markedly increased
  - » Evaluation for anticoagulants is part of the lupus reflex panel

Int J Lab Hematol. 2010; 32:132-8.
Lab Hematol. 2009; 15(4):45-48.

History and Structure of ARUP Panel

- Initial testing begins with D-dimer, fibrinogen activity, and LA reflex panel
  - » LA reflex includes
    - PT, aPTT, and dRVVT screen, with reflex to confirmatory assays if needed.
    - Steps to detect and neutralize anticoagulants
      - › At time of data collection, PT, thrombin time, and reptilase time to identify anticoagulants; heparinase for drug neutralization
      - › Recent update to LA panel (effective 2/20/2024) now uses qualitative anti-Xa activity evaluation and thrombin time; Heparinase and DOAC-Stop are both now available options for drug neutralization

History and Structure of ARUP Panel

- Based on pattern of initial results together with provided history, pathologist selects appropriate reflex testing
  - » PT-based factor assays– factors II, V, VII, X
  - » aPTT-based factor assays– factors VIII, IX, XI, XII
    - Chromogenic factor VIII and Bethesda assays for factor VIII and IX also available
  - » Von Willebrand assays (activity and antigen)
  - » Fibrinogen antigen
  - » Mixing studies

Benefits of Pathologist-Directed Ordering

- Pathologist-directed algorithmic approaches to reflex testing have been described to provide added benefit in hemostasis/thrombosis testing[1, 2]
  - » Improved test selection
  - » Decrease in unnecessary orders and costs
  - » Comprehensive interpretation to mitigate risk of misdiagnosis
- Pathologist-directed algorithmic testing also shown to be effective in other areas of hematopathology
  - » Ancillary test ordering for bone marrow biopsies to allow specific WHO classification diagnoses[3]

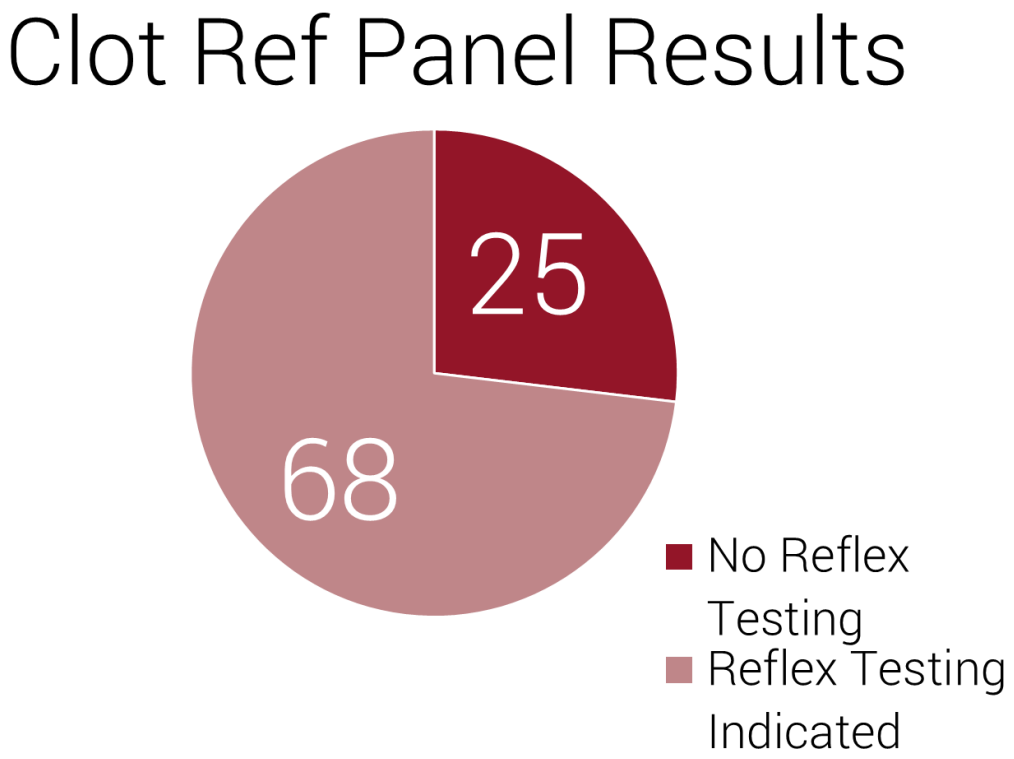
1. Kottke-Marchant K. Semin Thromb Haemost. 2014; 40:195-204.
2. Verna R, Velazquez AB, Laposata M. Ann Lab Med. 2019; 39:121-124.
3. Pearson LN, Miller JM, Lunde JH, et al. *Arch Pathol Lab Med*. 2019; 143(6):732-737.

ARUP experience with pathologist-directed prolonged clotting time panel

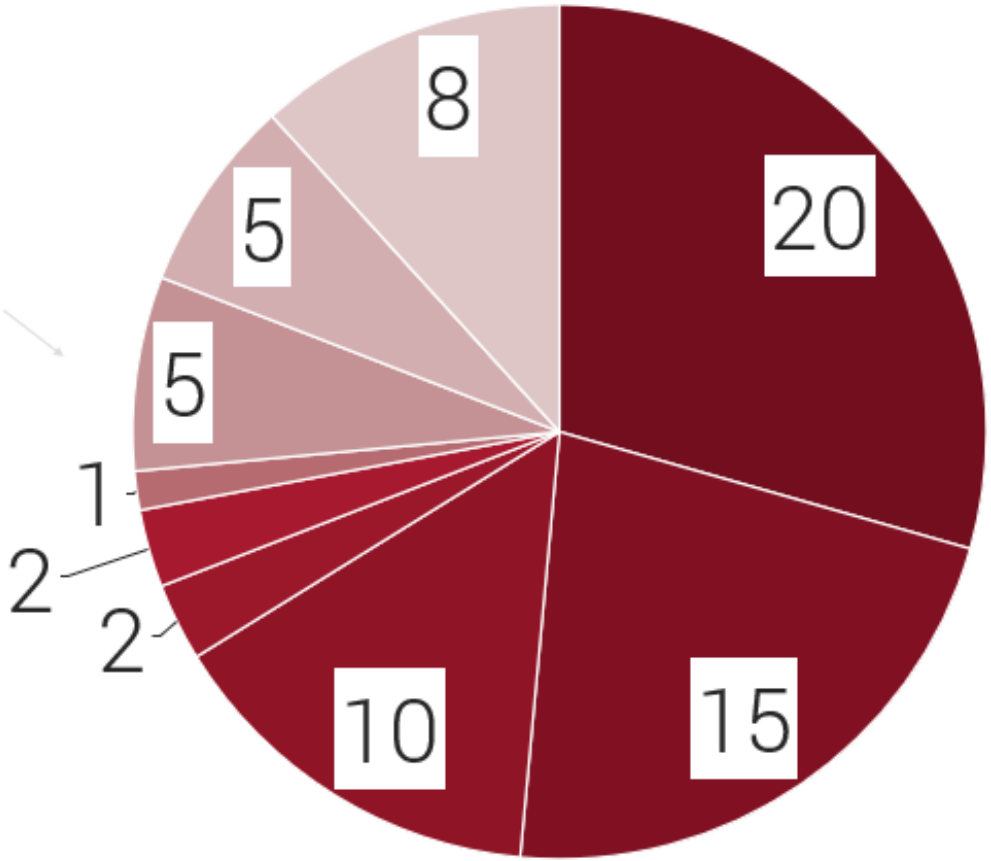
ARUP data presented in part in poster form at THSNA 2022
Moser KA, Lim MY, Smock KJ.

Data Review

- Evaluated ARUP prolonged clotting time reflex (Clot Ref) panels between 2017 and 2021
  - » 93 panels from 92 individual patients
- Why might there be cases with no reflex testing indicated?
  - » Variation between local and ARUP reagents
  - » Transient clotting time prolongation that resolved by the time reflex panel was ordered
  - » Preanalytic variables



Panels Requiring Reflex Testing—Findings



- Lupus anticoagulant (LA) +/- factor deficiencies
- Multiple factor deficiencies
- Single factor deficiency
- Possible von Willebrand disease
- High-titer factor VIII inhibitor
- Dysfibrinogenemia
- Drug effect as sole cause
- Normal clotting times, reflex tests based on patient history
- No cause identified with reflex testing

General Category of Reflex Testing Result	Specific Findings
Multiple factor deficiencies	3 DIC-like pattern
	2 liver disease-like pattern
	1 DIC versus liver disease-like pattern
	3 deficiencies of two intrinsic pathway factors (IX and XI, XI and XII, IX and XII)
	6 no specific pattern
Single factor deficiency	8 mildly decreased factor XII
	1 decreased factor II
	1 decreased factor X
Drug effect as sole cause of prolonged clotting times	1 heparin pattern
	1 vitamin K antagonist and heparin pattern
	1 direct Xa inhibitor pattern
	2 direct thrombin inhibitor versus excess heparin pattern
Normal clotting times, reflex testing based on patient history	1 mildly decreased factor VII insufficient to explain clinical bleeding
	1 mildly decreased factor X insufficient to explain clinical bleeding
	3 with no abnormalities of reflex tests

Patterns That Were Expected

- Lupus anticoagulants with or without factor deficiencies were the most common finding in cases with prolonged clotting times
  - » Factor deficiencies together with LA included
    - Multiple factor deficiencies, pattern unclear = 2
    - Possible vitamin K deficiency or warfarin = 2
    - Low factor XI activity = 1
    - Low factor X activity (insufficient to prolong clotting times) = 1
    - Mild decrease in factor XII activity and elevated factor VIII in setting of trauma = 1

Patterns That Were Expected

- Next most common pattern was multiple factor deficiencies
  - » Most common– no specific pattern (DIC, liver disease, and vitamin K deficiency in differential diagnosis)
  - » Also identified DIC-like (3) and liver disease-like (2) patterns, with one case pattern we were unable to distinguish between DIC and liver disease
  - » Some unexpected patterns in this category too (next slide)

Patterns That Were Surprising—And Why!

- Multiple factor deficiencies--2 intrinsic pathway factors
  - » Factor IX (75%) and XI (51%)
  - » Factor XI (48%) and XII (21%)
  - » Factor IX (71%) and XII (50%)
  - » Specimens did not show patterns of anticoagulant interference and did not have LA detected in confirmatory step
    - All had prolonged aPTT that did not correct in mixing study; possible weak LA not detected with our panel leading to factor assay interference
  - » Deficiencies were mostly mild

Patterns That Were Surprising—And Why!

- The most common single factor deficiency was factor XII
  - » At first, perhaps surprising as factor XII deficiency has an estimated prevalence of 1.5-3% in a healthy population[1]
  - » However, our panel is frequently used for evaluation of well outpatients with no history of bleeding and unexpected prolongation of clotting times—less surprising in that context
- Other single factor deficiencies included factor XI (1 case, 9% activity) and factor X (1 case, 49% activity)
  - » Neither case had a provided history form

1. Halbmayer WM, Haushofer A, Schon R, et al. Thromb Haemost. 1994; 71(1):68-72.

Reflex Tests Ordered Based on History

- Screening clotting times not prolonged in ARUP specimen, but history form indicated a history of bleeding
  - » Reflex testing ordered for these patients showed
    - Mildly decreased factor VII (71%), insufficient to explain clinical bleeding (1 case)
    - Mildly decreased factor X (72%), insufficient to explain clinical bleeding (1 case)
    - No abnormalities in any reflex tests, cause of prolonged clotting times unclear

Diagnoses Uncommonly Identified

- Factor VIII inhibitor
  - » Two cases, both high-titer inhibitors (18.6 BU and 115.0 BU)
  - » Both showed false positivity in aPTT-based but not dRVVT-based LA testing
- Dysfibrinogenemia
  - » 1 case
    - aPTT 50 s, aPTT 1:1 mix 42 s
    - PT 16.3 s, PT 1:1 mix 14.0 s
    - Thrombin time 28.1 s, Reptilase time 108 s
      - › No change in clotting times with heparinase
    - Fibrinogen activity 86 mg/dL, fibrinogen antigen 234 mg/dL
    - No other factor deficiencies identified
    - D-dimer 0.4 µg/mL FEU

Diagnoses Uncommonly Identified

- Possible von Willebrand disease cases (n = 2)
  - » Case 1
    - History of unexpected prolonged aPTT but no history of bleeding and no history of anticoagulation or transfusion
    - aPTT 60 s (32-48 s), aPTT 1:1 mix 43 s (corrected), PT 12.7 s, TT 15.6 s
    - vWF:Ag 46% (52-214%), vWF:RCo 67% (51-215%), factor VIII 30% (56-191%)
    - Normal factor IX, XI, and XII activities
    - Considerations include hemophilia A carrier (patient is female), possible type 2N vWD, or improper specimen handling
  - » Case 2
    - Personal and family history of epistaxis and menorrhagia
    - aPTT 45 s, PT 12.4 s
    - vWF:Ag 42%, vWF:Rco 24%; chromogenic factor VIII activity performed under separate accession was 75% (56-191%)
    - Follow-up testing included a normal VW multimer pattern and VWF collagen binding activity of 38% (50-203%)

How often did we see patterns suggesting anticoagulants?

- Heparin– no history forms
  - » 1 clear heparin pattern
    - Prolonged aPTT and thrombin time, normal reptilase time; aPTT corrected after heparin neutralization
  - » 2 DTI versus excess heparin beyond neutralizing capacity of our reagent
    - Marked aPTT prolongation, non-correction in aPTT mixing study, no correction post-heparinase, marked thrombin time prolongation with normal reptilase time

How often did we see patterns suggesting anticoagulants?

- Warfarin
  - 1 vitamin K antagonist/deficiency and heparin pattern (prolonged aPTT and TT with correction post-heparinase and normal reptilase time)
  - Decreased factor II (79%), VII (71%), X (61%) activity
  - History of bleeding and PT prolongation, anticoagulant information not provided
  - Vitamin K deficiency and heparin contamination from line draw?
  - Or missed documentation of anticoagulant therapy and patient has received both warfarin and UFH?
- What about DOACs?
  - 1 possible direct Xa inhibitor pattern in our study (and two possible DTI as noted above)
  - Prolonged PT and dRVVT with non-correction in mixing studies, no LA, nonparallelism in factor II, V, VII, X activity assays
  - No history form

How do our findings compare to other laboratories?

- Hazim AZ, Ruan GJ, Khodadadi RB et al. Int J Lab Hematol. 2022; 44:209-215.
  - » Review of 7 years (2010-2017) of prolonged clotting time panel evaluations in a national reference laboratory
    - 300 total patients
    - 106 outpatients from within the hospital system selected for further study
  - » 29/106 patients (27%) had normal clotting times in reflex panel; no additional testing performed
    - ARUP 25/93 panels (27%)

How do our findings compare to other laboratories?

- Findings in patients with prolonged clotting times

Finding	Hazim et al. (n = 77)	ARUP (n = 68)
Lupus anticoagulant	7	20
Vitamin K deficiency pattern	8	0
Liver disease-like pattern	15	2 (plus 1 DIC versus liver disease-like pattern)
Anticoagulant pattern	14 (4 additional with heparin contamination patterns)	5 (includes heparin pattern with no history submitted, could be therapeutic or contamination)

Hazim AZ, Ruan GJ, Khodadadi RB et al. Int J Lab Hematol. 2022; 44:209-215.
ARUP unpublished data.

How do our findings compare to other laboratories?

- Findings in patients with prolonged clotting times—single factor deficiencies

Finding	Hazim et al. (n = 77)	ARUP (n = 68)
Factor V deficiency	2	0
Factor VII deficiency	2	0
Factor VIII deficiency	3	0
Factor X deficiency	0	1
Factor XI deficiency	1	1
Factor XII deficiency	2	8

Hazim AZ, Ruan GJ, Khodadadi RB et al. Int J Lab Hematol. 2022; 44:209-215.
ARUP unpublished data.

How do our findings compare to other laboratories?

- Findings in patients with prolonged clotting times—specific factor inhibitors and other uncommon findings

Finding	Hazim et al. (n = 77)	ARUP (n = 68)
Factor VIII inhibitor	2	2
Factor XI inhibitor	1	0 (panel does not include factor XI Bethesda assay)
Dysfibrinogenemia	0	1
Possible von Willebrand disease	1	2

Hazim AZ, Ruan GJ, Khodadadi RB et al. Int J Lab Hematol. 2022; 44:209-215.
ARUP unpublished data.

How often did reflex testing fail to identify a cause of prolonged clotting times?

- ARUP
  - » 8 patients with prolonged clotting times and no cause identified with reflex testing
  - » 3 additional patients with no prolongation of ARUP clotting times and reflex testing ordered based on history
- Hazim et al.
  - » 7 patients with unclear etiology
- What could be going on?
  - » Possibly weak LA that were not detected by the panel methods

Hazim AZ, Ruan GJ, Khodadadi RB et al. Int J Lab Hematol. 2022; 44:209-215.
ARUP unpublished data.

Benefits of Panel

- Prevents additional phlebotomy
- Avoids blanket ordering of tests
- Strengthens conversations and collaborations with other physician and health care professional colleagues
  - » Provides support for colleagues who may not order specialized hemostasis tests often

Challenges of Panel

- Obtaining sufficient clinical history
  - » One-page form is not always completed and/or submitted
  - » Opportunity for systematic study of history form completion patterns to further understand our panel's performance
- Must state up front possible add-on tests, which prevents choosing any test available on our laboratory's menu

Conclusions

- Use of an algorithmic, reflexive strategy offers an efficient and effective plan for evaluating prolonged clotting times
- Pathologist-directed reflex ordering allows for a customized approach for each patient and particularly supports physicians who do not order hemostasis assays regularly

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